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Thrombocytopenia in critically ill patients with severe sepsis/septic shock: Prognostic value and association with a distinct serum cytokine profile☆

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ABSTRACT

Purpose: The purpose of the study is to evaluate the incidence, association with serum cytokine profile, and prognostic value of thrombocytopenia, in critically ill patients with severe sepsis/septic shock.
Methods: A cohort of 105 consecutive patients admitted in intensive care unit was included in our analysis. Serum levels of intercellular adhesion molecule, vascular cell adhesion molecule, interferon γ, interleukin 8, and soluble form of the urokinase-type plasminogen activator receptor (suPAR) were measured.
Results: Thrombocytopenia was observed in 53% of patients at the time of admission. Platelet counts showed a statistically significant negative correlation with serum levels of intercellular adhesion molecule, suPAR, and interleukin 8 ($P < .0001$). In multivariate analysis, high Acute Physiological and Chronic Health Evaluation II score, high serum suPAR, and low platelet counts were associated with increased mortality, and receiver operating characteristic curve analysis was used to determine the best cutoff value for mortality prediction. Each variable with a value above or below the predefined cutoff levels were given 1 point. Patients were categorized in risk groups based on total point score. High-risk (2-3), intermediate-risk (1), and low-risk (0 points) groups consisted of 43%, 22%, and 35% and 28-day mortality was observed in 69%, 26%, and 3% of the patients in each group, respectively.
Conclusion: Thrombocytopenia is associated with poor prognosis and a distinct serum cytokine profile.

1. Introduction

Sepsis is the clinical syndrome resulting from the inflammatory response of the host against various invading pathogens such as bacterial infections. Sepsis can be complicated by hemodynamic instability and multiorgan dysfunction and is commonly associated with hematological abnormalities. Patients with severe sepsis usually develop thrombocytopenia due to various reasons such as disseminated intravascular coagulation (DIC), drug-induced myelosuppression, heparin-induced thrombocytopenia (HIT), drug-induced immune destruction, hemodilution, massive transfusion, hemophagocytosis syndrome, and others [1]. However, in many instances, severe thrombocytopenia accompanies sepsis without an identifiable cause. In these cases, activation and consumption of platelets (PLTs) due to contact with a damaged endothelium have been postulated as the pathogenetic mechanism [2]. Irrespective of the underlying pathogenetic process, many studies have shown a negative impact of thrombocytopenia in sepsis outcome [3,4]. Serum cytokine profiling of patients with severe sepsis/septic shock is a valuable tool for better understanding of the underlying pathogenetic mechanism [5]. Patients with significant mortality risk as well as patients with high probability for evolution to organ dysfunction can be identified by the different cytokine profile [6].

Urokinase-type plasminogen activator is a protein involved in the conversion of plasminogen to the active fibrinolytic enzyme plasmin. Urokinase-type plasminogen activator is activated upon binding to its cognate receptor, which is expressed on the surface of various cell types including neutrophils, lymphocytes, monocytes, macrophages, and endothelial cells. After shedding from cell surface, urokinase-type plasminogen activator can be found in a soluble form in blood (suPAR) and other tissue fluids. Soluble form of the urokinase-type

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plasminogen activator receptor has a pleotropic function: except from regulating fibrinolysis, it is involved in inflammatory response and in various cell functions, including adhesion, migration, degradation of extracellular matrix, and angiogenesis [7]. Previous data suggest that serum suPAR is a predictor of outcome in critically ill patients and a marker of the degree of inflammation [8].

Serum levels of intercellular adhesion molecule (ICAM) 1, vascular cell adhesion molecule (VCAM) 1, and interleukin 8 (IL-8) have been used as markers of endothelial dysfunction which is a key factor in the pathogenesis of sepsis. Intercellular adhesion molecule 1, VCAM-1, and IL-8 are expressed on the surface of the endothelial cell in response to injury or activation by proinflammatory cytokines and result in leukocyte adhesion, increased transendothelial migration, and increased endothelial permeability [9,10].

The aim of this prospective, observational study was to describe the incidence, prevalence, and prognostic value of thrombocytopenia, in critically ill patients with severe sepsis and/or septic shock. We also studied the relationship between thrombocytopenia and patients' inflammatory response as expressed by the cytokines, suPAR, ICAM, and VCAM, IL-8, and interferon γ (IFN-γ).

2. Materials and methods

2.1. Patients

Our study included 105 consecutive patients with severe sepsis and/or septic shock treated in the intensive care unit (ICU) of our institute from October 2009 to September 2012. Exclusion criteria were HIV infection, mechanical ventilation for more than 72 hours prior to ICU admission, brain death, and no need for mechanical ventilation during ICU stay. This study was designed with the aim to examine the correlation between thrombocytopenia and patient's inflammatory response as well as the association with sepsis outcome. Therefore, patients with thrombocytopenia due to a known cause not related to sepsis such as (1) drug-induced thrombocytopenia, (2) hematologic malignancy, (3) HIT syndrome, (4) DIC directly related to a malignant disorder, and/or (5) pancytopenia due to previous administration of chemotherapy were excluded from the study. Systemic inflammation response syndrome, sepsis, severe sepsis, and septic shock were defined according to standard criteria [11]. Data collection was performed prospectively, and *outcome* was defined as ICU mortality. Briefly, 23 of 105 patients had undergone a previous operation the days before admission to ICU. Fifteen patients had an abdominal infection including peritonitis, diverticulitis, and cholangitis; 6 patients had bacteremia without an identifiable primary site of infection; 2 patients had bacterial endocarditis; 4 patients had central nervous system infection including meningitis and encephalitis; 58 patients had lower respiratory tract infection; 5 patients had soft tissue infection; and 15 patients had urinary tract infection.

Baseline clinical characteristics and laboratory results collected during the first 24 hours of admission were used for the estimation of the Acute Physiological and Chronic Health Evaluation (APACHE) II score [12]. The Sequential Organ Failure Assessment (SOFA) score recorded on the day of admission was used as a variable in statistical analysis [13].

Patients were categorized according to PLT count estimated at the time of admission. Platelet count at admission was considered as the lowest PLT count observed during the 3 first days after admission in ICU. *Thrombocytopenia* was defined as a PLT less than $150 \times 10^3/\mu L$. Thrombocytopenia was considered as mild ($100 \times 10^3/\mu L \le PLT < 150 \times 10^3/\mu L$), moderate ($50 \times 10^3/\mu L \le PLT < 100 \times 10^3/\mu L$), or severe ($PLT < 50 \times 10^3/\mu L$) depending on PLT counts. Written informed consent was given by all patients or by relatives of patients not able to give consent. Patient's characteristics are shown in more detail in Table 1.

2.2. Serum cytokines

Luminex xMAP technology (Luminex Corporation, Austin, TX) was used to measure serum concentrations of IFN-γ, IL-8, ICAM, and VCAM (ProcartaPlexTM immunoassays; eBioscience, Inc, San Diego, CA). The interassay and intraassay Coefficient of Variation (CV) in all assays performed were 3.5% to 8.5% and 4.6% to 12%, respectively. Serum suPAR levels were determined using a commercial enzyme-linked immunosorbent assay (suPARnostic Standard kit; ViroGates A/S, Birkerød, Denmark). This assay uses a double monoclonal antibody that measures circulating suPAR, including full-length and cleaved forms of the receptor. According to the standards provided by the manufacturer, a curve was constructed, and the results were expressed as nanograms per milliliter, in a range from 0.6 to 20.0 ng/mL. The intraassay and interassay CVs

Table 1
Patients characteristics

Clinical features	Normal PLT at admission (PLT >150000)	Thrombocytopenia at admission			Statistics
		Mild	Moderate	Severe	
No. of patients	49/105 (47%)	15/105 (14%)	7/105 (7%)	34/105 (32%)	–
Age, median, range	64 (21-85)	79 (45-89)	61 (53-82)	66 (28-90)	NS
Sex, male/female	32/17	7/8	6/1	17/17	NS
Surgery	11/49	4/15	1/7	7/34	NS
Malignancy	5/49	1/15	2/7	9/34	NS
Comorbidities[a]	33/49	12/15	3/7	19/34	NS
Diabetes	12/49	3/15	1/7	8/34	NS
COPD	10/49	2/15	0/7	5/34	NS
CHF/CAD	9/49	3/15	2/7	6/34	NS
CRF	7/49	2/15	1/7	3/34	NS
APACHE score, median, range	16 (5-24)	18 (8-31)	19 (15-22)	21 (9-30)	$P < .0001$
SOFA score (day 1), median, range	6 (1-12)	8 (6-14)	7 (5-11)	9 (5-16)	$P < .001$
Severe sepsis/septic shock	36/13	3/12	4/3	8/26	$P < .0001$
Bacteremia[b]	6/49	1/15	2/7	13/34	$P = .007$
Gram-negative sepsis[c]	34/49	2/15	3/7	17/34	NS
IFN-γ, median, range (pg/mL)	1.0 (1.0-71.0)	3.3 (1.0-47.3)	1.0 (1.0-23.7)	3.2 (1.0-126.4)	NS
ICAM, median, range (ng/mL)	80.1 (18.3-747.9)	123.3 (36.4-414.6)	175.7 (95.9-515.7)	226.1 (49.5-835.2)	$P < .0001$
IL-8, median, range (pg/mL)	7.2 (0.2-174.2)	14.9 (5.3-62.7)	23.1 (3.9-72.1)	38.1 (4.3-1140.4)	$P < .0001$
VCAM, median, range (ng/mL)	47.8 (8.0-428.5)	47.3 (25.9-648.7)	37.2 (11.3-339.8)	47.8 (5.6-1061.3)	NS
suPAR, median, range (ng/mL)	5.1 (1.4-10.5)	10.7 (3.5-113.9)	8.4 (6.6-33.7)	13.5 (3.3-57.6)	$P < .0001$
ICU mortality (%)	11/49 (22%)	10/15 (67%)	5/7 (71%)	31/34 (91%)	$P < .0001$

NS indicates nonsignificant.
[a] At least 1 of the following: diabetes, COPD, CHF/CAD, and CRF.
[b] At the time of admission.
[c] Infection due to gram-negative bacteria detected in blood, urine, bronchoalveolar lavage (BAL), or any other infected tissue at the time of admission.